DESORPTION, ACCUMULATION, AND ELIMINATION OF SEDIMENT-ASSOCIATED PHENANTHRENE AND BENZO[A]PYRENE TO A FRESHWATER OLIGOCHAETE

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ABSTRACT
A batch desorption method using isopropanol and electrolyte solution washing was used to desorb phenanthrene and benzo[a]pyrene inoculated sediments. Desorption kinetics and partition coefficient of the desorbed sediments were studied as well as their accumulation, and elimination by the oligochaete , *Ilyodrilus templetoni*. Desorption resistance was observed for sorbed phenanthrene but almost no resistance was observed for benzo[a]pyrene. The differences in desorption resistance were reflected in their accumulation by the worms. The steady state biota-sediment accumulation factor (BSAF) of reversibly sorbed phenanthrene was approximately two times higher than that of desorption-resistant phenanthrene, while no such effect was noted for benzo[a]pyrene. Benzo[a]pyrene was observed to accumulate more slowly than phenanthrene and was also subject to very low rates of elimination and negligible biotransformation. A model for uptake rate and extent was developed to describe data for both compounds.

RÉSUMÉ
Une méthode de désorption en réacteur batch à l'aide d'isopropanol et d'une solution d'électrolyte de lavage a été utilisée afin de désorber du phénanthrène et du benzo [a] pyrène inoculés dans des sédiments. La désorption et le coefficient de partition ont été étudiés de même que leur élimination par le vers oligochète, *Ilyodrilus templetoni*. Une résistance à la désorption a été observée pour le phénanthrène mais presque aucune résistance a été observée pour le benzo [a] pyrène. Les différences dans la résistance de désorption ont été reflétées dans l'accumulation par les vers. Le facteur d'accumulation entre la biote et le sédiment (BSAF) en régime stationnaire du phénanthrène réversiblement adsorbé, était approximativement deux fois plus élevé que celui du phénanthrène résistant à la désorption, aucun effet a été noté pour le benzo [a] pyrène. Le Benzo [a] pyrène s'est accumulé plus lentement que le phénanthrène en étant assujetti à des taux d'élimination très bas et de biotransformation négligeable. Un modèle a été développé pour décrire ces données pour les deux composés.

1. INTRODUCTION

Benzo[a]pyrene (BaP) and phenanthrene (PHE) are hydrophobic compounds with moderately large octanol water partition coefficients [1]. Upon entering aquatic systems, these compounds rapidly deposit into the sediment and physically adsorbs on the solid surface or partitions into the organic carbon fraction of the sediment. Many studies have demonstrated that the effect of the soil or sediment-associated contaminants on the receptor is not controlled by the total concentration of the contaminant, but instead by that fraction which is biologically available [2]. This fraction is usually defined as "bioavailability" of the contaminant. The bioavailability of sediment-associated BaP determines the potential hazard of this contaminant in the environment. Bioaccumulation tests are usually used to determine the bioavailability of sediment –associated contaminants and trophic transfer potential of the contaminant in the aquatic environment [3]. It is generally assumed that only desorbed contaminants are biologically available to organisms [4]. That is to say a contaminant must be first desorbed from sediment particles into pore water or an animal's digestive fluids before it can be taken up by the organisms. Therefore, sorption and desorption of the contaminant from sediment particles are very important factors that may determine the accumulation of a contaminant by a specific organism. Many laboratory results and field studies have demonstrated that some fraction of a contaminant desorbs quickly and reversibly, while desorption from a second fraction is limited in rate or extent, a phenomenon usually termed as desorption resistance [5,6,7,8]. Recent work suggests that desorption resistance may be the result of partitioning to soot or soot-like materials that exhibit a very large partition coefficient relative to natural organic matter [9,10,11]. The equilibrium pore water concentration of desorption-resistant compartment is much less than that expected by reversible desorption, which may, therefore, influence the uptake by sediment-dwelling organismsThe model presented by Kan et al [8] suggests that the effect of desorption resistance is minimal for highly hydrophobic compounds and that sediment-water partition coefficient is expected to be independent of concentration. The limited influence of desorption resistance for highly hydrophobic compounds is unexpected in that partitioning to condensed phase organic carbon would be expected to be greater for highly hydrophobic compounds too [11]. A lack of desorption resistance phenomena for highly hydrophobic compounds may be the result of a balance between the greater equilibrium partitioning and slow rate of approach to that equilibrium expected of highly hydrophobic compounds. Whether a compound exhibits desorption resistance or not is important because desorption resistance influence the bioaccumulation and effects related to the accumulation of

the compound. If highly hydrophobic compounds do not exhibit desorption resistance, (1) sediment-water partition coefficients should be independent of concentration (2) effects on organisms should not be reduced with the age of contaminated sediment and (3) toxicity should be well predicted by equilibrium partitioning theory.

Desorption of the adsorbed contaminant from the sediment particle may only be a necessary condition for the uptake of the contaminant. Many other factors, such as the route of uptake (water, sediment, or food) and the uptake efficiency from each source [2], the ability of the organism to metabolize the contaminant [4], and the relative contribution from each source [12,13], will influence the rate of uptake and steady-state accumulation of the contaminant. Assimilation efficiency (AE), which is defined as the fraction of the adsorbed products that is incorporated into the body tissue [14], is an important parameter in understanding and modeling contaminant accumulation and trophic transfer in the aquatic environment especially when sediment ingestion is the major uptake route. AE was also used in quantifying bioavailability of sediment-associated PHE and BaP by Penry et al[15]. Besides assimilation efficiency, elimination of the contaminants by active metabolic process and passive diffusion loss may also influence net accumulation, thus influencing the observed bioavailability of the contaminants.

In this study, a series of toxico-kinetic experiments was conducted on inoculated and isopropanol-desorbed sediments. Experiments were designed to measure the potential for desorption resistance, as well as the uptake kinetics, assimilation efficiency and elimination of these compounds in a deposit-feeding, freshwater oligochaete. The purposes of this study were to investigate bioavailability of sediment-associated BaP and PHE to a bulk deposit-feeding organism and to study the factors that may influence bioavailability.

2. MATERIALS AND METHODS

2.1 Sediment preparation and test organisms

The sediment used in this study was collected from Bayou Manchac, a freshwater bayou in Baton Rouge, Louisiana, USA, and passed through a 2mm sieve to remove debris and large particles. Phenanthrene (PHE) and Benzo[a]pyrene (BaP) were first plated on the wall of a glass jar, weighted sediment was added and tumbled for three weeks to ensure that the contaminants partitioned homogeneously to the matrices of sediment. At the same time, approximately 3000ppm-sodium azide was added to inhibit the active metabolism of phenanthrene by bacteria. To increase the analysis sensitivity, [^{14}C] phenanthrene (Chemsyn Science Laboratories, Kansas, USA) was added together with unlabeled phenanthrene (Sigma, St Louis, MO, USA). The total BaP concentration in the sediment was primarily unlabeled BaP (Sigma, St Louis, MO, USA), supplemented with radio-labeled [^{3}H] BaP (American Radiolabeled Chemicals Incorporation, St Louis, MO, USA)

Sediment was then desorbed by an isopropanol and electrolyte solution (0.01M NaCl, 0.01M $CaCl_2 \times 2H_2O$) with a ratio of 1:1. After each batch of desorption, sediment was centrifuged at 4200rpm for 25 minutes and washed 3 to 4 times with electrolyte solution to remove the residual isopropanol and sodium azide. Total PHE and BaP concentration and the proportion of reversible to desorption-resistant contaminants are controlled by the number of batch desorptions and the time periods of each desorption. Tomson and Kan (unpublished manuscript) have shown that desorption with isopropanol solution allows rapid removal of reversibly sorbed material, and the resulting isotherm tracks multiple batch-desorptions with water. Sediment loading was analyzed by both high-performance liquid chromatography (HPLC) and liquid scintillation counting (LSC). The analysis by LSC is simpler, more direct, and more sensitive. Thus analysis of worms' body burden and pore water concentrations was based on the results of LSC. oligochaete, was used in this study because of its ease to culture, ability to withstand handling stress and high tolerance to contaminants [16].

2.2 Measurement of uptake kinetics

Measurement of the uptake kinetics was conducted in 50-ml glass tubes. In each tube, 20 or 15 worms of similar sizes were exposed to approximately 50 g wet sediment (moisture content ~40%). Time to apparent steady state (ts) of the uptake was observed to be a matter of days for PHE and of the order of a month for BaP. Steady state was assumed to be reached if there was no statistically significant difference of the biota-sediment accumulation factors in the last two exposures. At each sampling time, three tubes from each sediment were sacrificed, the worms were sieved from the sediment, survivors were enumerated and allowed to purge their digestive system for 6 hours in clean artificial pond water (0.5 mM NaCl, 0.2 mM NaHCO3, 0.05mM KCl, 0.4 mM CaCl2). Radio-labeled BaP or PHE accumulated in the worms' tissue was counted immediately on a Beckman LS 6000IC liquid scintillation counter (Beckman-Counter, Fullerton, CA, USA), and the other 6 worms in each tube were frozen for lipid analysis.

2.3 Measurement of assimilation efficiency

Assimilation efficiency was measured using the pulse-chase feeding technique described by Selck et al [17], and based on the direct measurement of the radiolabeled BaP or PHE ingested and remaining in tissue after complete egestion. Twenty-four worms were first exposed to 15 ml radiolabeled sediment for 40 minutes (less than or equal one gut-passage time). Then, worms were gently but quickly taken out from the radiolabeled sediment, flushed with water to remove isotope adsorbed on the surface of the worms' body, and divided into two groups: from each replicate, 9 worms were placed in an ingestion group and 15 worms in a depuration group. Worms in the ingestion group were subjected to LSC immediately, in groups of three worms. The average of the total count normalized by worms' weight of the three sub-samples was taken as the ingested sediment concentration. The worms in the depuration group were moved to the unlabeled sediment to purge the

ingested materials. Five worms were counted together to ensure that high counts could be obtained after depuration, and the average of the count was taken as the BaP or PHE concentration assimilated into worms' tissue Ft. Then, assimilation efficiency was calculated as Ft/FI.

Worms in the depuration group were transferred to clean sediment allowed to depurate for 4, 8, 24 and 48 hr to determine when gut clearance occurred. At each sampling time, 15 worms were removed and tissue concentration was measured by LSC. The fraction of mass remaining after depuration was calculated at each period, and complete egestion was estimated from the profile of this fraction. Assimilation efficiency was calculated as the fraction of the remaining body burden at upon complete egestion, which was estimated to be 4 hr.

2.4 Elimination and biotransformation

In the elimination experiment, *I. templetoni* were exposed to the contaminated sediment for 7 days. After placement in artificial pond water (9 hours for BaP and 20 hours for PHE) to allow gut clearance, individuals were transferred to clean sediment and analyzed for body burden at different periods. Data were fitted by a first- order decay model and elimination rate constant was determined from the model. Elimination determines the total loss of BaP or PHE from worms' tissue including both active and passive processes. A biotransformation experiment was conducted to define the metabolism of BaP and PHE by the worms or active loss of these two compounds following the procedure of Milward et al [18].

3. DATA ANALYSIS

Accumulation data of BaP were analyzed using both an equilibrium partitioning bioaccumulation model defined by biota-sediment accumulation factor (BSAF)and a first- order kinetic model [19]. Ratio of lipid normalized tissue concentration to organic carbon normalized sediment concentration was calculated at each exposure period, and the ratio at steady state defined the biota-sediment accumulation factor. Only steady state biota-sediment accumulation factor of PHE was calculated due to the complicated kinetics observed for the uptake of PHE. When sediment is the source compartment, a kinetic model of uptake can be written as:

$$\frac{dC_t}{dt} = k_s C_s - k_e C_t \qquad (1)$$

Where

Ct, BaP concentration accumulated in worms' tissue, dpm/mg dry tissue.
Cs, sediment concentration, dpm/mg dry sediment
ks, uptake rate coefficient from the sediment, g sediment/g worm• hr-1
ke, elimination rate constant, hr-1

Assuming sediment concentration is constant during exposure, and integrating Eqn.1:

$$C_t = \frac{k_s \cdot C_s}{k_e}(1 - \exp(-k_e t)) \qquad (2)$$

Fitting the uptake kinetics data with Eqn.2 using a non-linear regression technique provided by SigmaPlot (SPSS Science, Chicago, IL,USA) gave the estimated value of uptake rate coefficient ks and elimination rate constant ke. Biota-sediment accumulation factor predicted from the kinetic model as time approaches infinity:

$$BSAF_{pred} = \frac{k_s}{k_e}\frac{f_{oc}}{f_{lipid}} \qquad (3)$$

Elimination from worms was described by a first order decay model:

$$C_t = C_{t=0} \bullet e^{(-k_e t)} \qquad (4)$$

4. RESULTS

4.1 Sediment characteristics and desorption isotherms

Sediment characteristics, including BaP or PHE concentration and activity, organic carbon content (foc), and organic carbon normalized sediment-water partition coefficients (Koc) were monitored. Organic carbon content (foc) of the sediment was reduced approximately 9% during the first batch desorption but was essentially constant in the subsequent desorptions. Desorption isotherms of BaP and PHE was determined based on the measured solid concentration and the pore water concentration of the tested sediments. Strong desorption resistance was detected at low concentrations for PHE, but .no obvious desorption resistance was observed for BaP as the desorption isotherm of BaP was identical to that estimated by reversible desorption (Fig. 1).

4.2 Bioaccumulation

Tissue concentration of BaP and PHE was higher than the sediment concentration even after short exposures , and body burden increased with increasing sediment concentration. Uptake of BAP was almost linear during the short exposure (less than 2 days), and an apparent steady state was reached after approximately one month. Although biota-sediment accumulation factor is defined at steady state, the same normalization method was used at each exposure period of BaP to observe the change of the normalized accumulation before the biota-sediment accumulation factor was obtained. Rapid uptake was observed with PHE but, due to unknown experimental factors, the uptake decreased after a short time followed by attainment of steady state within 7 days.

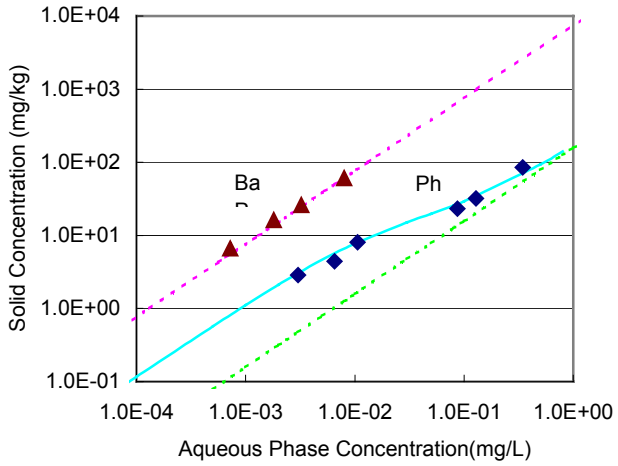

Figure 1 – Desorption isotherms of PHE and BaP

The uptake kinetics of BaP was fit by a first-order kinetic model (Eqn.2). The BaP biota-sediment accumulation factor calculated from the estimated uptake rate coefficient and elimination rate constant (Eqn.3) were 1.48 and 1.50 for two sediments, which were very close to the biota-sediment accumulation factor measured at steady state. PHE BSAFs were independent of the sediment concentration but strongly dependent on whether the contaminants exhibited desorption resistance. BSAFs of the six sediments fell into two categories. BSAFs of three "partially" desorbed sediments that contained PHE that partitioned as expected by linear, reversible sorption exhibited a higher level with BSAF of 1.2(±0.32). BSAFs of three sediments with a PHE partition coefficient that deviated from the linear reversible desorption curve (Figure 1) exhibited a BSAF approximately half of that exhibited by the reversibly desorbed phenanthrene.

4.3 Assimilation efficiency

In the depuration group, the fraction of BaP remaining in the worms' tissue decreased from 100% with all ingested sediments still in worms' digestive system at the beginning of the depuration period to 80% after 4 hr in unlabeled sediment, and this value became almost stable after that. The rapid loss in the first 4 hr was probably due to digestive clearance (purging), and the subsequent slow changes in concentrationwere likely due to elimination from the worms' tissue. Assimilation efficiency as defined by the fraction of body burden remained at the conclusion of sediment egestion was approximately 80% for BaP, indicating that 80% of ingested sediment-associated BaP would be taken up by the organisms during a single gut-passage.

A similar analysis with PHE showed that PHE assimilation efficiency was limited to about 50% of that ingested, and no significant difference was observed for reversibly sorbed and desorption-resistant phenanthrene.

4.4 Elimination and biotransformation

Both elimination of phenanthrene and benzo[a]pyrene followed a first-order pattern(r^2>0.88) although BaP showed a much slower elimination rate. The elimination rate of phenanthrene fitted by the first- order model (Eqn.2) was 0.042 hr^{-1}, which was approximately 7 times faster than the elimination of benzo[a]pyrene, whose elimination rate was 0.0066 hr^{-1}. Approximately 15% of the parent phenanthrene was metabolized during a 7d-exposure, and less than 6% of the parent benzo[a]pyrene was metabolized by the worms during a 38 d-exposure, indicating that benzo[a]pyrene resists metabolism by *I. templetoni.*.

5. DISCUSSION

Our empirically derived measure of the desorption-isotherm of benzo[a]pyrene and phenanthrene demonstrated that there was no significant resistance in the desorption of sediment-associated BaP while significant desorption resistance was noted for PHE. This result is consistent with the prediction of the biphasic model of Kan et al [8], which states that desorption resistance, as indicated by increases in apparent partition coefficient, becomes less pronounced when the hydrophobicity of a compound increases, and when Koc of an organic contaminant is greater than the predicted organic carbon based partition coefficient of the desorption-resistant fraction. Kan et al. suggested an organic carbon based partition coefficient to the desorption resistant fraction of $10^{5.53\pm0.48}$ mL/g. The apparent independence of the partitioning to the desorption- resistant fraction is unexpected but may be due to the slower kinetics of sorption and desorption of more sorbing compounds.

The observed inverse relationship between partition coefficients and BSAFs was consistent with a two-stage process of uptake into the organism's lipid, but only the first stage,partitioning to sediment pore water or worms' digestive fluid would be limited by desorption resistance. In previous research, we developed a model that predicts bioaccumulation (BSAF) based on a contaminant's effective partition coefficient from a variety of laboratory results with phenanthrene, pyrene and literature data in which sorbents are used to control pore water concentration [20]. These data suggested that bioaccumulation is well predicted by partitioning into the pore water phase. That is, the BSAF is reduced by desorption resistance inversely proportional to the increase in the sediment-water partitioning coefficient.

$$BSAF = \frac{\frac{C_t}{f_{lipid}}}{\frac{C_S}{f_{Toc}}} = \frac{\frac{K_{lw}}{f_{lipid}}}{\frac{K_{sw}}{f_{Toc}}} = K_{oc} / K_{oc}^{res} \cdot BSAF^{lab} \qquad (4)$$

As shown in Figure 2, the accumulation that is predicted from the effective partition coefficient is in good agreement with a variety of laboratory and literature data including the accumulation of BaP.

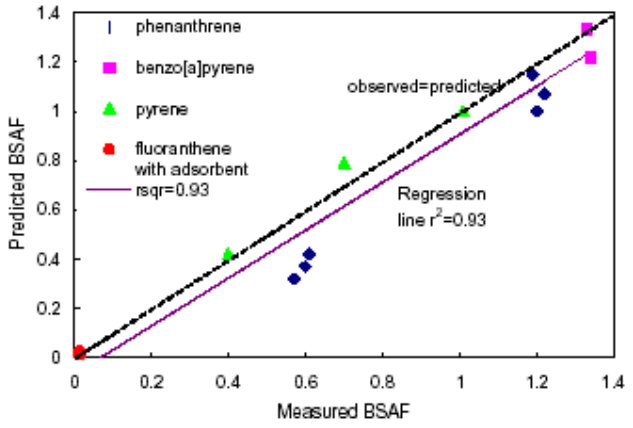

Figure 2 – Measured BSAFs vs that predicted from the effective partition coefficient, i.e. the porewater paradigm

This finding is consistent with the paradigm that the effective pore water concentration may control the bioaccumulation of hydrophobic contaminants by deposit-feeding organisms for a given compound. This appears to be true even though in the case of BaP, literature results have shown that the primary route of exposure is ingestion of sediment for BaP [21,22,23,]. In this case, the two-stage uptake includes the partitioning to the worms' gut fluid and uptake from gut fluid to worms' tissue. The surfactants in the gut fluid may enhance the release of the contaminant and potentially increase measures of single gut passage assimilation efficiency and the rate of the uptake. The gut fluid, as an intermediate phase, however, cannot influence the ultimate partitioning between the two phases: sediment and worm's tissue. Thus, the ultimate or steady state bioaccumulation of the BaP and phenanthrene were observed to be independent of the measured assimilation efficiency and controlled only by partitioning in sediment, water, and lipid systems. As with any three-phase system in physical equilibrium, partitioning between any two of these phases defines the partitioning to the third phase. This conclusion has been developed only with soft bodied deposit-feeding benthic oligochaetes that are not expected to metabolize PAHs and thus contaminant uptake is largely associated with partitioning. The applicability of the model to other benthic organisms and other contaminants needs to be explored.

6. CONCLUSIONS

Sediment-associated phenanthrene and benzo[a]pyrene were available to deposit-feeding, freshwater oligochaete, *Ilyodrilus templetoni*. No obvious resistance, as measured by desorption isotherm, was observed for benzo[a]pyrene, which resulted in almost identical normalized accumulation at the high and low concentrations of the desorbed sediments. Significant desorption resistance was noted for phenanthrene and this was reflected in the steady state accumulation of this compound. This was consistent with the paradigm that effective pore water concentration controls the uptake of hydrophobic organic contaminant in the deposit-feeding organisms.

The assimilation efficiency thus defines the rate of uptake by sediment ingestion while the steady state accumulation under the conditions of this study is defined by simple partitioning. Therefore, the accumulation of BaP and PHE was observed to be independent of the measured assimilation efficiency. The pore water concentration in the sediment can then be used to define the steady state accumulation despite ingestion being the primary route of uptake. Desorption resistance reduces bioavailability and bioaccumulation of these partitioning contaminants by reducing pore water concentration and producing a corresponding reduction in steady state accumulation. This conclusion would be complicated by metabolic processes that might occur with other compounds or in other organisms that would introduce fate processes other than simple partitioning.

7. ACKNOWLEGEMENT

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